The effects of organic and inorganic phosphorus concentration on the acid phosphatase activity of ectomycorrhizal fungi

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Kinetic constants (K_m and V_{max}) were determined for surface and extracellular soluble acid phosphatases produced by two ectomycorrhizal fungi (*Cenococcum geophilum* Fr. and *Entoloma sericeum* (Bull. ex Merat) Quel.) grown in axenic culture at 2 or 50 μM KH_2PO_4 or sodium inositol hexaphosphate. Results for cultures supplied inorganic phosphorus were similar to those supplied organic phosphorus: surface V_{max} estimates were significantly greater for 2 than for 50 μM grown isolates. The presence of constitutive extracellular soluble phosphatase activity resulted in the appearance of inorganic phosphate in media initially supplied with organic phosphorus, suggesting substrate hydrolysis in excess of phosphate uptake. No consistent relationship was found between apparent K_m estimates and phosphorus treatments. The two species had surface phosphatase V_{max} values differing by as much as two orders of magnitude. The magnitude of the response to phosphorus treatment differed among isolates. The response of phosphatases to changes in phosphorus at concentrations comparable with soil solution phosphorus supports the hypothesis that levels of available soil phosphorus may control ectomycorrhizal phosphatase production or activation.

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Les auteurs ont déterminé les constantes cinétiques (K_m et V_{max}) de phosphatases acides, soit liées à la surface ou soit extracellulaires et solubles, produites par deux champignons ectomycorhiziens (*Cenococcum geophilum* Fr. et *Entoloma sericeum* (Bull. ex Merat) Quél.) cultivés axéniquement en présence de KH_2PO_4 ou d'inositol hexaphosphate aux concentrations 2 et 50 μM. Les résultats sont identiques pour les deux sources de P, inorganique et organique, offertes aux cultures; les V_{max} pour la surface sont significativement plus élevées à 2 qu'à 50 μM. La présence de phosphatase extracellulaire constitutive soluble et active se manifeste par l'apparition de P inorganique dans le milieu ayant reçu au départ le P sous forme organique, ce qui suggère que l'hydrolyse du substrat excède l'absorption des phosphates. Aucune relation claire n'apparaît entre les valeurs K_m estimées et les traitements P. Les deux espèces montrent des valeurs V_{max} pour la phosphatase de surface qui diffèrent entre elles par près de deux ordres de grandeur. L'importance de la réaction aux traitements P diffère selon les isolats. Les réactions des phosphatases aux fluctuations du P à des concentrations comparables à celle de la teneur de la solution du sol, confirme l'hypothèse que les teneurs des sols en P soluble peuvent contrôler la production ou l'activation des phosphatases ectomycorhiziennes.

[Traduit par la revue]

Introduction

The increase in phosphorus accumulation and improvement of phosphorus status of higher plants caused by symbiotic association with mycorrhizal fungi have been well documented (Hatch 1937; Harley and McCready 1950; Rhodes and Gerdemann 1975). The principal factor limiting phosphorus uptake in most soils is thought to be the diffusion rate of inorganic phosphate to the root surface (Sutton and Gunary 1969; Nye 1977). Enhanced phosphorus uptake by mycorrhizae has been attributed primarily to mycorrhizal hyphae increasing the absorptive area of the root and expanding the total soil volume exploited for nutrient uptake (Sanders and Tinker 1971; Bowen *et al.* 1975).

The presence on ectomycorrhizal fungi of nonspecific surface acid phosphatases capable of hydrolyzing a number of organic phosphorus compounds has invited speculation that mycorrhizae may contribute to the phosphorus nutrition of host plants in another way: this enzyme-catalyzed hydrolysis may produce inorganic phosphate from organic sources otherwise unavailable to the higher plant (Bartlett and Lewis 1973). The largest fraction of total soil phosphorus is organic, with much of that occurring as inositol phosphates (Dalal 1977); an organism's ability to use this phosphorus pool, especially in soils low in inorganic phosphate, might provide a considerable adaptive advantage.

The regulation of mycorrhizal acid phosphatases by inorganic phosphate has been investigated by a number of researchers. Field studies by Alexander and Hardy (1981) showed an inverse correlation between extractable soil inorganic phosphorus and acid phosphatase activity on mycorrhizal roots of Sitka spruce. Some culture studies with ectomycorrhizal isolates, although done at phosphorus concentrations higher than those found in most soils, have corroborated these observations (Calléja *et al.* 1980; Lacaze 1983). However, Dighton (1983), who surveyed the phosphatase activity of 10 fungal isolates grown in inorganic and organic phosphorus, concluded that phosphatase production was independent of the levels of inorganic or organic phosphate at which the fungi were grown. More recent work with *Pisolithus tinctorius* has confirmed that increased phosphatase activity is associated with phosphorus deficiency (Berjaud and d'Auzac 1986; Mousain and Salsac 1986). Similar results were found in studies done with *Suillus granulatus* (Bousquet *et al.* 1986) and with a number of fungi that form *Pinus* mycorrhizae (Doumas *et al.* 1986).

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The purpose of this study was to investigate the regulation of surface and soluble acid phosphatases by orthophosphate and inositol hexaphosphate for isolates of *Cenococcum* and *Entoloma* from arctic ecosystems. Phosphorus is thought to be a limiting nutrient in many arctic tundra ecosystems, not because the total phosphorus capital is low but because much of it is tied up in organic forms (Chapin *et al.* 1978). Average solution inorganic phosphate concentrations may be as low as 0.5 μM, with soil solution organic phosphorus 23 times higher (Barel and Barsdate 1978). Mycelia of ectomycorrhizal fungi isolated from tundra soils, plants, or fruiting bodies have active surface acid phosphomonoesterases (Antibus *et al.* 1981), but whether these enzymes are produced or activated in response to the presence of organic phosphorus and absence of inorganic phosphorus is not known.

To examine the regulation of acid phosphomonoesterase activity of ectomycorrhizal isolates of *Cenococcum geophilum* and *Entoloma sericeum* from phosphorus-poor arctic and temperate environments, we determined the kinetic parameters for phosphatases of fungi grown at two concentrations of inorganic or organic phosphorus. We hypothesized that changes in either inorganic or organic phosphorus within the concentration range of natural soil solutions would affect both mycelial growth and the kinetic constants (apparent K_m and V_{max}) of surface and extracellular soluble acid phosphomonoesterases.

Materials and methods

Four isolates of the ascomycete *Cenococcum geophilum* Fr. and one of the basidiomycete *Entoloma sericeum* (Bull. ex Merat) Quel. were grown in liquid medium of various organic or inorganic phosphate concentrations. Two of the *C. geophilum* isolates (one from *Pinus virginiana* in Maryland and the other from *Salix rotundifolia* at Cape Simpson, Alaska) were assayed for surface and extracellular soluble phosphatase activity at 12-day intervals for 48 days. The *Entoloma* isolate (from Barrow, Alaska) and the other two *C. geophilum* isolates (from Barrow and Eagle Creek, Alaska) were assayed once: at 24 days for the *Entoloma* and Eagle Creek *C. geophilum* isolates and at 37 days for the slower growing Barrow isolate. All isolates except the Maryland *C. geophilum* previously had formed typical ectomycorrhizae with the dwarf arctic willow *Salix rotundifolia* (Antibus *et al.* 1981). The effects of pH, temperature, and substrate concentration on these enzymes have been documented (Antibus *et al.* 1986).

Plugs 5 mm in diameter were cut from the outer edge of mycelia growing on plates of Hagem's medium (Antibus *et al.* 1986), divided in half, and transferred to 250-mL Erlenmeyer flasks containing 50 mL Hagem's medium amended as follows: a 4 mM citrate buffer (pH 5.6) was used in place of phosphate buffer, 3.8 mM KCl was added as a potassium source, and malt was omitted. Phosphorus was provided as orthophosphate (KH_2PO_4) or phytic acid (sodium inositol hexaphosphate) at concentrations of either 2 or 50 μM. In addition to this amount of phosphorus, each flask contained "pre-incubation" phosphorus, which was added to the flask in the agar plugs used as inocula. This was at most 13 μg per flask, increasing the total phosphate concentration to a maximum of 10 and 60 instead of 2 and 50 μM. Because this phosphorus was consistent across treatments, we refer to the treatments as 2 and 50 μM throughout the paper, the amount of inorganic or organic phosphorus in addition to the amount of pre-incubation phosphorus in each flask. Five flasks of each phosphorus treatment, each inoculated with five half plugs, were analyzed at each sampling date.

The cultures were incubated at 20°C in the dark. The pH of the medium after autoclaving was approximately 5.0 and declined with time after inoculation to a minimum of 4.5 at day 48.

Surface phosphatase activity was measured by removing individual mycelial mats from flasks, rinsing them three times with distilled water, blotting them on absorbent paper, and then incubating each mycelial mat in 2 mL of buffered *p*-nitrophenyl phosphate (pNPP) solution (pH 5.5) at 20°C for 3 h on a mechanical shaker. The buffer used (modified universal buffer) was prepared according to Ho and Zak (1979). Substrate concentrations used were 1, 5, 10, 25, and 50 mM pNPP. Five mycelial mats, one from each of the five replicate flasks, were assayed at a single concentration; 25 mycelial mats were used for each kinetic assay. After incubation, 0.5 mL from each tube was added to 0.5 mL 0.5 N NaOH and stirred using a Vortex mixer. The optical density at 410 nm was measured on a Gilford model 250 spectrophotometer. Assayed mycelial mats were rinsed in distilled water, frozen in liquid nitrogen, lyophilized, and weighed on a Cahn 26 electrobalance. The weight of the agar in the inoculum plugs is included in the weights presented in the Results section. Extracellular soluble acid phosphatase activity was assayed in a similar manner using aliquots of culture media. Residual inorganic phosphate in the medium at each sampling date also was measured (Bencini *et al.* 1983).

Phosphatase activity pH profiles were generated to determine whether across-treatment comparisons would be affected by pH optimum differences. Mycelial mats of the Maryland isolate (at 69 days) were incubated in saturating pNPP concentrations (50 mM). Both substrate and buffer were adjusted to pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0.

Enzyme activity is expressed in micromoles product (*p*-nitrophenol) per hour per milligram dry mass of mycelia. This "surface activity" is comparable with the "accessible" activity of Calléja *et al.* (1980), which they found to be representative of phosphatase activity associated with the cell wall and plasmalemma outer surface. Soluble activity is expressed in micromoles product per hour per milligram dry mass of mycelia, based on total volume of culture medium and total dry mass of mycelia in each flask. Estimates of apparent K_m and V_{max} were determined from Eadie–Hofstee transformations of the enzyme activity data. Analysis of variance and Duncan's multiple comparison tests (SPSS Inc. 1983) were used to analyze the data.

Results

Surface phosphatase V_{max} estimates for the two isolates assayed at 12-day intervals are presented in Fig. 1. The isolates responded similarly to both inorganic (P_i) and organic (P_o) phosphorus: V_{max} estimates were significantly higher for fungi grown in 2 μM P_i or P_o than for those grown in 50 μM P_i or P_o. V_{max} estimates increased first for 2 μM P_o grown fungi and continued to be higher through the end of the 48 days. The Maryland isolate of *C. geophilum* responded most to phosphorus deficiency; by day 24, the 2 μM P_i and P_o treatments resulted in surface phosphatase V_{max} values at least six times higher than those of the 50 μM P_i and P_o treatments, and by day 48 they were higher by 12 to 18 times.

Surface phosphatase V_{max} estimates for two of the three isolates assayed at one point in time also were higher for fungi grown in 2 μM P_i or P_o than for those grown in 50 μM P_i or P_o, although the differences for *E. sericeum* were not statistically significant (Table 1). *Entoloma sericeum*, assayed at day 24, had V_{max} values between one and two orders of magnitude greater than did the *C. geophilum* isolates at day 24. V_{max} values determined for the Barrow isolate of *C. geophilum* at 37 days exhibited no pattern and no significant differences (Table 1). The poor growth exhibited by this isolate may account for the high variability in data.

The effect of phosphorus form and its concentration on growth rate for the Maryland and Cape Simpson isolates of *C. geophilum* is shown in Fig. 2. For the first 24 days, there were few significant differences in growth for either isolate. After 48 days, dry mass of samples grown in 50 μM P_i or P_o

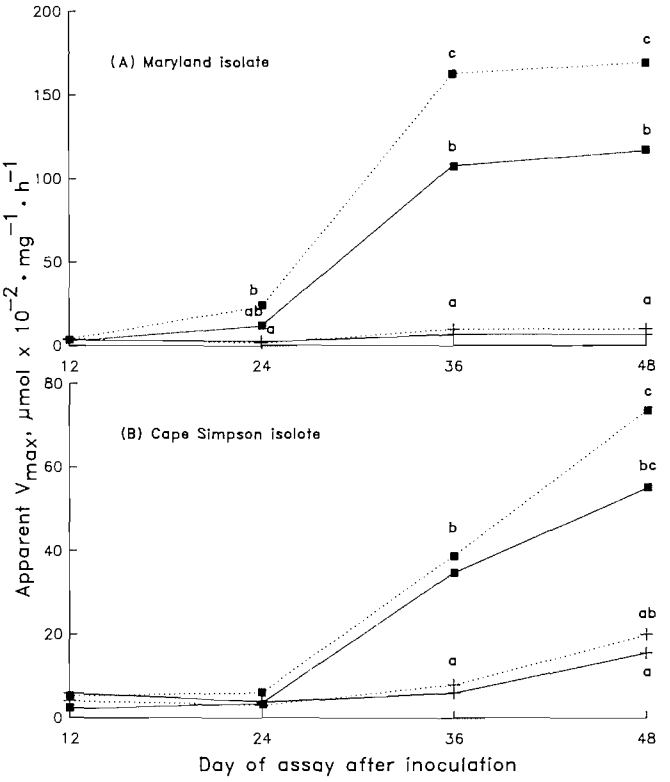

FIG. 1. V_{max} estimates (μmol × $10^{-2} \cdot mg^{-1} \cdot h^{-1}$) determined by Eadie–Hofstee transformations of acid phosphatase activity data for *C. geophilum*. ——, P_i; . . . . , P_o; ■, 2 μM; +, 50 μM; $n = 5$. (A) Maryland isolate. (B) Cape Simpson isolate. Points labeled with the same letter within each assay date are not significantly different ($p \leq 0.05$).

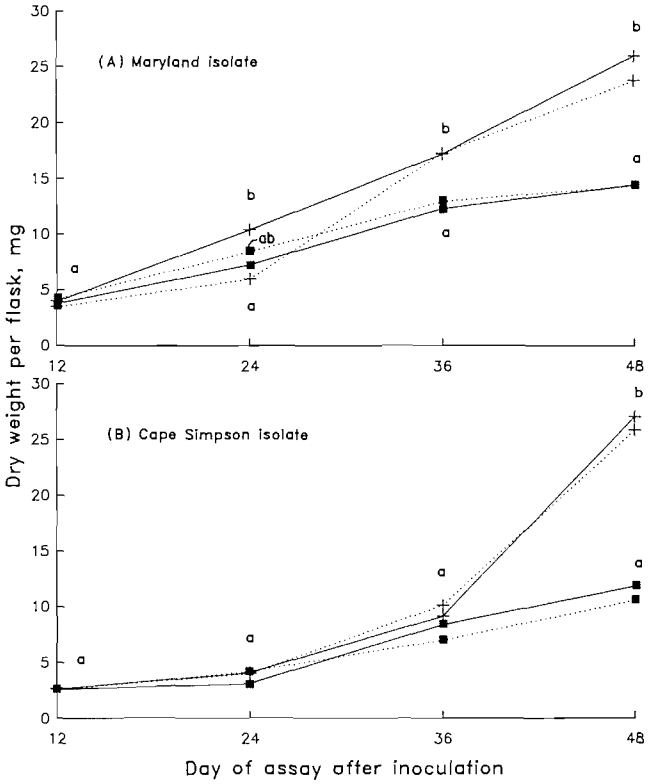

FIG. 2. Total dry mass per flask (mg) for *C. geophilum*. ——, P_i; . . . . , P_o; ■, 2 μM; +, 50 μM; $n = 5$. (A) Maryland isolate. (B) Cape Simpson isolate. Points labeled with the same letter within each assay date are not significantly different ($p \leq 0.05$).

was approximately twice as high as for those grown in 2 μM P_i or P_o. The Barrow isolate grew slightly faster in 50 μM P_i than in other treatments (Table 2). Differences for *E. sericeum* samples were not statistically significant, and growth of the Eagle Creek isolate also was not affected by P treatments (Table 2).

Extracellular soluble phosphatase activity based on dry mass generally decreased with time. By day 48, activity was greater for the 2 μM P_i and P_o supplied cultures than for the 50 μM P_i or P_o cultures for both the Maryland and Cape Simpson isolates (Fig. 3). For the other two isolates of *C. geophilum*, there were no clear trends (Table 1). Apparent V_{max} values for *E. sericeum* soluble phosphatases were significantly higher for 2 μM P_o grown samples than for the other three treatments (Table 1).

The relationship between extracellular soluble phosphatase activity and P_i concentration in either inorganic or organic phosphorus medium at each assay date is presented for the Maryland and Cape Simpson isolates grown at 50 μM P (Fig. 4). Media prepared with inositol hexaphosphate had no measurable P_i at the beginning of the incubation period, but P_i concentrations increased with initial growth and soluble phosphatase activity increases. Inorganic phosphate concentration in the P_i treatment flasks, however, continued to decrease throughout the fungal growth period. Patterns of the level of soluble phosphatase activities were similar for both treatments.

We found no consistent relationship between apparent K_m estimates and P treatments. Values listed in Table 1 are representative of those found for all isolates assayed. K_m values determined for the Maryland isolate were significantly higher for mycelia grown in 2 μM P_i or P_o than for those grown in 50 μM P_i or P_o on day 48. For the Cape Simpson isolate, however, the reverse was true. Surface phosphatase K_m values of *E. sericeum* and the Eagle Creek and Barrow isolates of *C. geophilum* showed no significant differences (Table 1). There were no consistent trends in K_m estimates for extracellular soluble phosphatases for any of the isolates. All K_m estimates for soluble phosphatases were lower than those for surface phosphatases (see Table 1).

Surface phosphatase activities measured from pH 2.5 to 6.0 for the Maryland isolate were optimal at pH 5.0, with no statistically significant differences between pH 5.0 and 5.5, regardless of the source or concentration of P (Fig. 5).

Discussion

Both inducible and repressible phosphatases occur in a variety of plants and fungi. Although a number of compounds inhibit or activate the enzyme (Buchanan *et al.* 1979; Akiyama *et al.* 1981; Arnold and Garrison 1979), inorganic phosphate is probably the most important factor in the regulation of repressible acid phosphatases *in vivo* (Woolhouse 1969; Patni *et al.* 1977; Arnold and Garrison 1979; McLachlan 1980; Boutin *et al.* 1981; Fernandez *et al.* 1981; Dracup *et al.* 1984).

Our data indicate that inorganic phosphate is an important regulator of surface phosphatase activity for ectomycorrhizal fungi as well. The activity of surface acid phosphatases of the isolates we examined was affected by changes in solution phosphorus concentration at less than 100 μM. We found a consistent inverse relationship between phosphorus concentra-

TABLE 1. V_{max} estimates. Maximum velocity (μmol · mg^{-1} · h^{-1}) and Michaelis–Menten constant (K_m, mM) determined by Eadie–Hofstee transformations of acid phosphatase activity data for *C. geophilum* (Eagle Creek: $V_{max} \times 10^2$ for both surface and soluble values; Barrow: $V_{max} \times 10^2$ for surface, $V_{max} \times 10$ for soluble values) and for *E. sericeum* (soluble V_{max} values $\times$ 10). The Barrow isolate was assayed at day 37 and the others at day 24

Treatment	V_{max}, surface	V_{max}, soluble	K_m, surface	K_m, soluble
Entoloma sericeum				
2 μM P_i	4.69*a**	8.31*a*	19.12*a*	1.98*a*
50 μM P_i	1.82*a*	3.95*a*	16.77*a*	1.51*a*
2 μM P_o	5.39*a*	15.07*b*	20.66*a*	1.88*a*
50 μM P_o	3.16*a*	6.67*a*	24.28*a*	1.54*a*
Cenococcum geophilum				
Eagle Creek				
2 μM P_i	2.60*a*	6.78*a*	9.15*a*	4.90*a*
50 μM P_i	1.34*b*	6.68*a*	6.58*a*	5.01*a*
2 μM P_o	2.43*a*	6.65*a*	10.11*a*	5.38*a*
50 μM P_o	1.33*b*	7.87*a*	7.08*a*	5.36*a*
Barrow				
2 μM P_i	3.55*a*	1.75*a*	10.60*a*	4.85*a*
50 μM P_i	3.24*a*	1.60*a*	12.80*a*	5.87*a*
2 μM P_o	3.41*a*	1.86*a*	13.37*a*	5.40*a*
50 μM P_o	3.09*a*	1.89*a*	12.57*a*	5.48*a*

*Numbers followed by the same letter within each column at each isolate are not significantly different ($p \leq 0.05$).

TABLE 2. Dry mass per flask (mg) at two concentrations of P_i and P_o for *C. geophilum* (Eagle Creek and Barrow isolates) and *E. sericeum*. The Barrow isolate was assayed at day 37 and the others at day 24

Treatment	*Entoloma sericeum*	*C. geophilum*, Eagle Creek	*C. geophilum*, Barrow
2 μM P_i	16.92*a**	4.96*a*	2.95*a*
50 μM P_i	21.92*a*	4.83*a*	3.84*b*
2 μM P_o	15.31*a*	5.43*a*	2.93*a*
50 μM P_o	18.33*a*	4.61*a*	2.96*a*

*Numbers followed by the same letter within each column are not significantly different ($p \leq 0.05$).

tion in the medium and phosphatase activity. In some cases, the V_{max} values for isolates grown at 2 μM phosphorus were 18 times higher than those for isolates grown at 50 μM phosphorus. Our findings support those of earlier culture studies done with different species and higher concentrations of inorganic phosphate (Calléja *et al.* 1980; Lacaze 1983; Berjaud and d'Auzac 1986; Bousquet *et al.* 1986; Mousain and Salsac 1986).

We cannot draw conclusions about the effect of organic phosphate on surface or soluble phosphatase production. The isolates examined in this study responded to differences in phosphate concentration but less clearly to differences in phosphate source. These results agree with those of Mousain and Salsac (1986), who found that the growth and inorganic phosphate accumulation of *Pisolithus tinctorius* were similar whether they were grown in inorganic or organic phosphate. In our study, the presence of extracellular soluble phosphatases in the culture medium resulted in the hydrolysis of inositol hexaphosphate to available inorganic phosphate by extracellular phosphatases, thus preventing the mycelia in inositol hexaphosphate supplied flasks from experiencing an inorganic phosphate deficiency. The presence of relatively large concentrations of inorganic phosphate (higher than the amount of preincubation P introduced when flasks were inoculated) in flasks to which only inositol hexaphosphate was added supports this hypothesis (see Fig. 4). Apparently hydrolysis rates were exceeding phosphate uptake rates. The early increase in surface phosphatase V_{max} values for the 2 μM P_o grown isolates of *C. geophilum* (Fig. 1) may reflect a phosphate deficiency caused by increasing difficulty in the hydrolysis of lower inositol phosphates (e.g., inositol tetraphosphate) by extracellular enzymes.

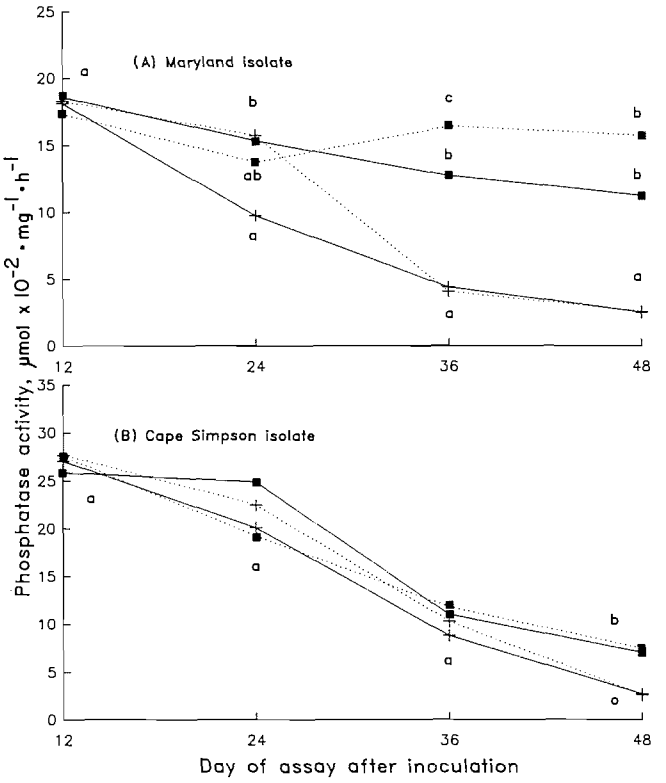

FIG. 3. Extracellular soluble phosphatase activity determined at 50 mM pNPP for *C. geophilum*, based on total dry mass of mycelia in each flask (μmol $\times$ 10^{-2} · mg^{-1} · h^{-1}). ——, P_i; . . . . , P_o; ■, 2 μM; +, 50 μM; $n = 5$. (A) Maryland isolate. (B) Cape Simpson isolate. Points labeled with the same letter within each assay date are not significantly different ($P \leq 0.05$).

The nearly identical levels of soluble phosphatase activity measured at 12 days suggest a constitutive enzyme, especially since early soluble phosphatase activity differed more among isolates than among treatments. Beever and Burns (1980) suggest that the secretion of phosphatases into the external environment is an advantage only if the enzyme stays close enough to the cell to ensure that the inorganic phosphate released can be taken up. The extensive hyphal network formed by mycorrhizae in soil may help to ensure that this condition is met.

The higher activities for surface and extracellular soluble phosphatases in the low-phosphorus flasks at later assay dates suggest that as phosphorus concentrations in either mycelia or media decrease, activity and (or) production of both surface and extracellular phosphatases increase. Apparently our "low P" treatment, 2 μM, provided sufficient P only until some of that phosphorus initially supplied (in liquid medium and in the agar plugs used to inoculate) was depleted. The fact that dry mass differences due to phosphate source (inorganic or organic) were not significant at either concentration provides

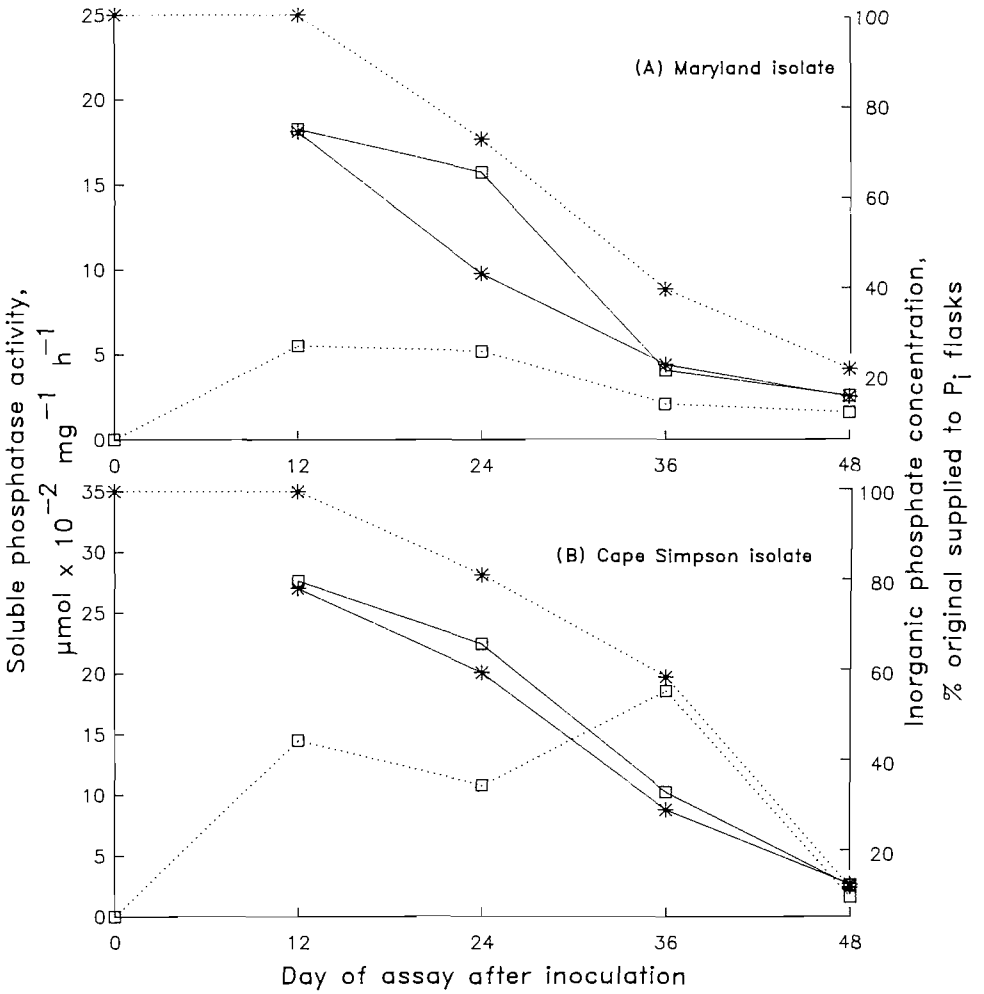
(A) Maryland isolate
(B) Cape Simpson isolate
Soluble phosphatase activity, μmol x 10⁻² mg⁻¹ h⁻¹
Inorganic phosphate concentration, % original supplied to Pi flasks
Day of assay after inoculation

FIG. 4. Inorganic phosphorus concentration (as percentage of 50 μM) (. . . .) and extracellular soluble phosphatase activity (——) in 50 μM P_i and P_o treatment flasks for *C. geophilum*. *, 50 μM P_i; □, 50 μM P_o; $n = 5$. (A) Maryland isolate. (B) Cape Simpson isolate.

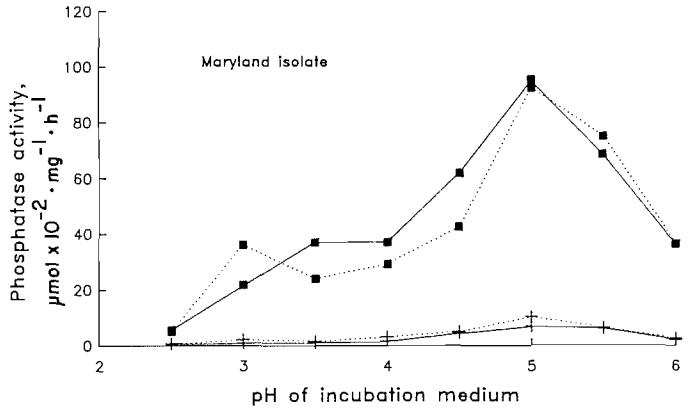

FIG. 5. Phosphatase activity (μmol $\times 10^{-2} \cdot mg^{-1} \cdot h^{-1}$) for *C. geophilum*, Maryland isolate. ——, P_i; . . . . , P_o; ■, 2 μM; +, 50 μM; $n = 3$.

evidence that phosphatase production was induced before growth limitations began: surface and soluble enzyme activities increased significantly even when there were no significant differences between growth in organic and inorganic phosphate supplied flasks. Analysis of internal metabolic and storage phosphorus pools might provide more definitive information about whether it is inorganic phosphate deficiency in the medium or in the fungus that is more important in the regulation of surface phosphatase production and activity.

The apparent K_m estimates determined in this study provide no clear evidence for the production of new phosphatase isozymes in response to phosphorus treatment. Berjaud and d'Auzac (1986) demonstrated that *Pisolithus tinctorius* had two major acid phosphatases, which appeared to be the same whether the fungus was grown at 2 mM KH_2PO_4 or with no phosphate at all. In our study, estimates of K_m were higher by three orders of magnitude than typical soil solution organic phosphate concentrations. A K_m larger than the concentration of substrate usually available may allow for faster hydrolysis of occasional large additions of substrate (Price and Stevens 1982). In this case, high K_m values may be simply the result of using an artificial substrate (pNPP) at unrealistically high concentrations. The K_m values of extracellular soluble phosphatases were consistently lower than those for surface phosphatases, reflecting differences between bound and soluble enzymes.

The difference in magnitude of response to phosphorus treatments between the Maryland and Cape Simpson, Alaska, isolates of *C. geophilum* may indicate adaptation to different edaphic conditions. The difference between the *Cenococcum* isolates and *E. sericeum*, which had surface V_{max} values two orders of magnitude larger than those of *Cenococcum*, sup-

ports the suggestion that ectomycorrhizal fungi differ in their ability to enhance phosphorus uptake (Ho and Zak 1979; Doumas *et al.* 1986).

The importance of organic phosphate as a phosphorus source for plants has been investigated by a number of researchers (Tate 1984). Anderson *et al.* (1974) suggested that the high stability of inositol hexaphosphate in soils and its resistance to decomposition were responsible for its relatively high concentration in many soils. Greenwood and Lewis (1977) found that species of soil yeasts able to utilize sodium and calcium salts of inositol hexaphosphate were unable to use the insoluble aluminum and iron salts; these insoluble forms may predominate in many soils. The demonstration that mycorrhizal fungi can utilize some forms of this organic phosphate compound as a sole source of phosphorus (Theodorou 1968; Mitchell and Read 1981; Dighton 1983; Mousain and Salsac 1986) suggests that mycorrhizae may contribute to the decomposition of inositol hexaphosphate and that it in turn may contribute to the phosphorus nutrition of mycorrhizal plants. The fact that the activity and V_{max} values of surface and extracellular phosphatases change in response to phosphorus concentration at the micromolar level supports the hypothesis that available soil phosphorus plays a role in the regulation of ectomycorrhizal phosphatase activity or production.

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AKIYAMA, T., UCHIMIYA, H., and SUZUKI, H. 1981. Gibberellic acid-stimulated *de novo* synthesis of a particular isozyme of acid phosphatase in wheat half-seeds. Plant Cell Physiol. **22**: 1029–1034.

ALEXANDER, I. J., and HARDY, K. 1981. Surface phosphatase activity of Sitka spruce mycorrhizas from a serpentine site. Soil Biol. Biochem. **13**: 301–305.

ANDERSON, G., WILLIAMS, E. G., and MOIR, J. O. 1974. A comparison of the sorption of inorganic orthophosphate and inositol hexaphosphate by six acid soils. J. Soil Sci. **25**: 51–62.

ANTIBUS, R. K., CROXDALE, J. G., MILLER, O. K., and LINKINS, A. E. 1981. Ectomycorrhizal fungi of *Salix rotundifolia*. III. Resynthesized mycorrhizal complexes and their surface phosphatase activities. Can. J. Bot. **59**: 2458–2465.

ANTIBUS, R. K., KROEHLER, C. J., and LINKINS, A. E. 1986. The effects of external pH, temperature and substrate concentration on acid phosphatase activity of ectomycorrhizal fungi. Can. J. Bot. **64**: 2383–2387.

ARNOLD, W. N., and GARRISON, R. G. 1979. An iron (III)-activated acid phosphatase in *Saccharomyces rouxii*. J. Biol. Chem. **254**: 4919–4924.

BAREL, D., and BARSDATE, R. J. 1978. Phosphorus dynamics of wet coastal tundra soils near Barrow, Alaska. *In* Environmental chemistry and cycling processes. *Edited by* D. C. Adriano and I. L. Brisbin, Jr. DOE Symp. Ser. 45. pp. 516–537.

BARTLETT, E. M., and LEWIS, D. H. 1973. Surface phosphatase activity of mycorrhizal roots of beech. Soil Biol. Biochem. **5**: 249–257.

BEEVER, R. E., and BURNS, D. J. W. 1980. Phosphorus uptake, storage and utilization by fungi. Adv. Bot. Res. **8**: 127–219.

BENCINI, D. A., WILD, J. R., and O'DONOVAN, G. A. 1983. Linear one-step assay for the determination of orthophosphate. Anal. Biochem. **132**: 254–258.

BERJAUD, C., and D'AUZAC, J. 1986. Isolement et caractérisation des phosphatases d'un champignon ectomycorhizogène typique, *Pisolithus tinctorius*. Effets de la carence en phosphate. Physiol. Veg. **24**: 163–172.

BOUSQUET, N., MOUSAIN, D., and SALSAC, L. 1986. Influence de l'orthophosphate sur les activités phosphatases de *Suillus granulatus* en culture *in vitro*. Physiol. Veg. **24**: 153–162.

BOUTIN, J. P., PROVOT, M., and ROUX, L. 1981. Effect of cycloheximide and renewal of phosphorus supply on surface acid phosphatase activity of phosphorus deficient tomato roots. Physiol. Plant. **51**: 353–360.

BOWEN, G. D., BEVEGE, D. I., and MOSSE, B. 1975. Phosphate physiology of vesicular–arbuscular mycorrhizas. *In* Endomycorrhizas. *Edited by* F. E. Sanders, B. Mosse, and P. B. Tinker. Academic Press, London, New York, and San Francisco. pp. 241–260.

BUCHANAN, B., CRAWFORD, N. A., and WOLOSIUK, R. A. 1979. Activation of plant acid phosphatases by oxidized glutathione and dehydroascorbate. Plant Sci. Lett. **14**: 245–251.

CALLÉJA, M., MOUSAIN, D., LECOUVREUR, B., and D'AUZAC, J. 1980. Influence de la carence phosphatée sur les activités phosphatases acides de trois champignons mycorhiziens: *Hebeloma edurum* Metrod., *Suillus granulatus* (L. et Fr.) O. Kuntze et *Pisolithus tinctorius* (Pers.) Coker et Couch. Physiol. Veg. **18**: 489–504.

CHAPIN, F. S., III, BARSDATE, R. J., and BAREL, D. 1978. Phosphorus cycling in Alaskan coastal tundra: a hypothesis for the regulation of nutrient cycling. Oikos, **31**: 189–199.

DALAL, R. C. 1977. Soil organic phosphorus. Adv. Agron. **29**: 83–117.

DIGHTON, J. 1983. Phosphatase production by mycorrhizal fungi. Plant Soil, **71**: 455–462.

DOUMAS, P., BERJAUD, C., CALLÉJA, M., COUPÉ, M., ESPIAU, C., and D'AUZAC, J. 1986. Phosphatases extracellulaires et nutrition phosphatée chez les champignons ectomycorhiziens et les plantes hôtes. Physiol. Veg. **24**: 173–184.

DRACUP, M. N. H., BARRETT-LENNARD, E. G., GREENWAY, H., and ROBSON, A. D. 1984. Effect of phosphorus deficiency on phosphatase activity of cell walls from roots of subterranean clover. J. Exp. Bot. **35**: 466–480.

FERNANDEZ, M. P., SUAREZ RENDUELES, M. P., and GASCON, S. 1981. Synthesis and regulation of molecular forms of yeast acid phosphatase. Cell Mol. Biol. **27**: 703–707.

GREENWOOD, A. J., and LEWIS, D. H. 1977. Phosphatases and the utilisation of inositol hexaphosphate by soil yeasts of the genus *Cryptococcus*. Soil Biol. Biochem. **9**: 161–166.

HARLEY, J. L., and MCCREADY, C. C. 1950. The uptake of phosphate by excised mycorrhizal roots of the beech. New Phytol. **49**: 388–397.

HATCH, A. B. 1937. The physical basis of mycotrophy in the genus *Pinus*. Black Rock For. Bull. **6**: 1–168.

HO, I., and ZAK, B. 1979. Acid phosphatase activity of six ectomycorrhizal fungi. Can. J. Bot. **57**: 1203–1205.

LACAZE, B. 1983. Localisation cytochimique des activités phosphatasiques acides de champignons mycorhiziens développés sur milieu complet ou carencé en phosphate. Can. J. Bot. **61**: 1411–1414.

MCLACHLAN, K. D. 1980. Acid phosphatase activity in intact roots and phosphorus nutrition in plants. II. Variations among wheat roots. Aust. J. Agric. Res. **31**: 441–448.

MITCHELL, D. T., and READ, D. J. 1981. Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of *Vaccinium macrocarpon* and *Rhododendron ponticum*. Trans. Br. Mycol. Soc. **76**: 255–260.

MOUSAIN, D., and SALSAC, L. 1986. Utilisation du phytate et activités phosphatases acides chez *Pisolithus tinctorius*, basidiomycète mycorhizien. Physiol. Veg. **24**: 193–200.

NYE, P. H. 1977. The rate-limiting step in plant nutrient absorption from soil. Soil Sci. **123**: 292–297.

PATNI, N. J., DHAWALE, S. W., and AARONSON, S. 1977. Extracellular phosphatases of *Chlamydomonas reinhardi* and their regulation. J. Bacteriol. **130**: 205–211.

PRICE, N. C., and STEVENS, L. 1982. Fundamentals of enzymology. Oxford University Press, Oxford. pp. 124–125.

RHODES, L. H., and GERDEMANN, J. W. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. **75**: 555–561.

SANDERS, F. E., and TINKER, P. B. 1971. Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas. Nature (London), **233**: 278–279.

SPSS INC. 1983. SPSSx user's guide. McGraw-Hill Book Company, New York.

SUTTON, C. D., and GUNARY, D. 1969. Phosphate equilibria in soil. *In* Ecological aspects of the mineral nutrition of plants. *Edited by* I. H. Rorison. Blackwell Scientific Publications, Oxford. pp. 127–134.

TATE, K. R. 1984. The biological transformation of P in soil. Plant Soil, **76**: 245–256.

THEODOROU, C. 1968. Inositol phosphates in needles of *Pinus radiata* D. Don and the phytase activity of mycorrhizal fungi. Proceedings of the Ninth International Congress on Soil Science, Adelaide, **3**: 483–493.

WOOLHOUSE, H. W. 1969. Differences in the properties of the acid phosphatases of plant roots and their significance in the evolution of edaphic ecotypes. *In* Ecological aspects of the mineral nutrition of plants. *Edited by* I. H. Rorison. Blackwell Scientific Publications, Oxford. pp. 357–380.